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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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GENENCOR INTERNATIONAL, INC.
ATTENTION: LEGAL DEPARTMENT
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EXAMINER	
LEFFERS JR, GERALD G	

ART UNIT	PAPER NUMBER
1636	

DATE MAILED: 11/05/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/954,737	BRON ET AL.	
	Examiner	Art Unit	
	Gerald G Leffers Jr., PhD	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 September 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *3-11* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3-11* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *15 February 2002* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Receipt is acknowledged of an amendment, filed 9/22/03, in which the pending claims were amended to delete non-elected subject matter (e.g. claims 1-2, and embodiments drawn to the LipA secretion signal). Claims 3-11 are pending in the instant application.

Election/Restrictions

Applicant's election of Group III (claims 3-11) in the paper filed 9/22/03 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Sequence Compliance

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 because sequences were set forth that lack sequence identifiers, no computer readable format (CRF) was filed, no paper sequence was filed and no attorney statement was filed. These sequences include, for example, **those presented in the Tables (e.g. Table 1)**. If the Sequence Listing required for the instant application is identical to that of another application, a letter may be submitted requesting transfer of the previously filed sequence information to the instant application. For a sample letter requesting transfer of sequence information, refer to MPEP § 2422.05. Additionally, it is

often convenient to identify sequences in figures by amending the Brief Description of the Drawings section (see MPEP § 2422.02).

Applicants are required to comply with all of the requirements of 37 CFR 1.821 through 1.825. Any response to this office action that fails to meet all of these requirements will be considered non-responsive. The nature of the noncompliance with the requirements of 37 C.F.R. 1.821 through 1.825 did not preclude the continued examination of the application on the merits, the results of which are communicated below.

Information Disclosure Statement

Receipt is acknowledged of information disclosure statements filed 7/1/02 and 4/9/03. The corresponding PTO Form 1449's have been mailed along with the instant office action.

Specification

The specification is objected to in that the claim for priority in the first sentence of the specification is inaccurate. The first sentence of the instant specification currently claims benefit under 35 U.S.C. 119(e) to U.S. Application No. 60/233,610, filed September 18, 2001. According to the records of the USPTO, the correct filing date is September 18, 2000. Correction is required.

Tables I and IV (pages 56 and 59, respectively) are objected to because the shaded portion of each table is so dark as to make it difficult to determine what the actual amino acids sequences are for each of the given polypeptides. It would be remedial to

amend the specification to include tables that still manage to show the regions of similarity or identity among the different polypeptides, but that do not obscure the actual sequences.

Drawings

New corrected drawings are required in this application because each of the figures comprising a picture of a gel and/or Western blot, as well as the figure providing sequence comparisons for the different Tat proteins, are unclear and nearly illegible (i.e. Figures 1, 4-6, 8-13). This makes it difficult to assess the information provided by each of the cited figures with regard to patentability of the pending claims. Applicant is advised to employ the services of a competent patent draftsperson outside the Office, as the U.S. Patent and Trademark Office no longer prepares new drawings. The corrected drawings are required in reply to the Office action to avoid abandonment of the application. The requirement for corrected drawings will not be held in abeyance.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 3-11 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one

skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Each of the rejected claims comprises the phrase "a PhoD signal sequence". The specification does not appear to limit this term to the phosphodiesterase obtained from *Bacillus subtilis* that is described in the instant specification (e.g. Tables I & 4). Thus, the term "PhoD" can be reasonably be read to encompass any enzyme or protein in the art that is termed "PhoD", and/or any phosphodiesterase that is expressed in a given host cell (e.g. any other prokaryotic or eukaryotic source) and is in any way analogous to the *B. subtilis* PhoD described by the instant specification.

The crux of the instant invention is the discovery of a novel twin-arginine translocation system in *B. subtilis* that is useful for the expression and secretion of polypeptides in *B. subtilis* and *E. coli*. The basis for the system is the signal sequence obtained for the PhoD protein of *B. subtilis* that comprises a novel "twin-arginine" translocation motif. The instant specification provides data concerning the characterization of the TatAd/Cd-dependent transport of PhoD into culture medium by *B. subtilis* (e.g. pages 32-33). The specification provides a relevant working example wherein the *B. subtilis* components (i.e. heterogeneously expressed TatAd/Cd proteins and a PhoD signal sequence) are used to express and secrete proteins (i.e. PrePhoD or SP_{PhoD}-LacZ fusion) in *E. coli*.

The specification does not, however, provide sufficient basis for one of skill in the art to envision other embodiments of a PhoD signal sequence that would be operative in their system for TatAd/Cd-dependent secretion of proteins in bacterial cells. The specification provides the amino acid sequence for the *B. subtilis* PhoD signal peptide

(apparently that shown in Table 1 on page 56 of the instant specification), as well as comparisons between the PhoD signal sequence and others obtained from B. subtilis (e.g. Table I) or to similar sequences found in sequence databases (e.g. Table IV). The specification does not teach, however, what alterations can be made to the *B. subtilis* PhoD signal sequence such that it will still function to direct translocation of a heterologous sequence across the cell membrane. Even for the known *B. subtilis* PhoD signal sequence, the specification describes experiments where it is shown that there is a unique requirement for the Tat proteins TatAd and TatCd be expressed in order for PhoD-dependent translocation across the cell membrane to occur in *B. subtilis* or *E. coli*. The amino acid residues of the *B. subtilis* PhoD signal sequence required for the TatAd/Cd interaction are not described (e.g. page 33, first paragraph). The specification teaches that it is unclear why *B. subtilis* PhoD requires the presence of TatCd for efficient secretion, especially since the twin-arginine motif (R-R) for PhoD appears to be similar to other known R-R motifs (e.g. page 28). Therefore, the instant specification does not provide a rational basis for one of skill in the art to envision those embodiments of a "PhoD" signal sequence that will function to transport proteins across a bacterial cell membrane.

The prior art appears to be silent with regard to the construction of fusion proteins comprising a "PhoD" signal sequence such that the fusion protein is capable of secretion across a host cell membrane. Thus, the prior art does not offset the deficiencies of the instant specification with regard to describing the broadly claimed genus of "PhoD" signal sequences.

Given the very broadly claimed genus of "PhoD" signal sequences, and the lack of a means provided in the prior art or instant specification for those sequences embraced by the claims that will function to mediate translocation of a protein across the cell membrane, the skilled artisan would not have been able to envision a sufficient number of specific embodiments that would describe the broadly claimed genus of such "PhoD" signal sequences. Therefore, the skilled artisan would reasonably have determined applicants were not in possession of the claimed invention.

Claims 3-11 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for embodiments wherein the host cell is a bacterial cell overexpressing the *B. subtilis* TatAd/Cd proteins and wherein the PhoD signal sequence is the *B. subtilis* PhoD signal sequence, does not reasonably provide enablement for any other embodiments. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention: The invention is complex, comprising the use of a given bacterial secretion signal to direct the secretion of a fusion protein comprising the signal

sequence across the host cell membrane to the periplasmic space (e.g. gram negative bacteria such as *E. coli*) and/or culture medium (e.g. gram positive bacteria such as *B. subtilis*). As such, the claimed invention involves numerous protein-protein interactions that are dependent upon the ability of the proteins involved in the secretion mechanism to recognize one another and function to translocate a protein across the barrier of a host cell membrane.

Breadth of the claims: The breadth of the claims exacerbate the complexity of the invention in that the claims encompass the use of *any* "PhoD" signal sequence (e.g. homologs or variants) obtained from *any* source (e.g. *Zymomonas*) to direct the translocation of any fusion polypeptide across the cell membrane of literally *any* host cell (e.g. Claim 7 encompasses bacterial, yeast, human cells, etc.). As such, the "PhoD" signal sequence must be able to function in any cell type with at least one cellular apparatus for translocation (e.g. components of a Sec or Tat pathway).

Guidance of the specification/Working examples: The instant specification provides data concerning the characterization of the TatAd/Cd-dependent transport of PhoD into culture medium by *B. subtilis* (e.g. pages 32-33). The specification provides a relevant working example wherein the *B. subtilis* components (i.e. heterogeneously expressed TatAd/Cd proteins and a PhoD signal sequence) are used to express and secrete proteins (i.e. PrePhoD or SP_{PhoD}-LacZ fusion) in *E. coli*. The specification provides the amino acid sequence for the *B. subtilis* PhoD signal peptide (apparently that shown in Table 1 on page 56 of the instant specification), as well as comparisons between the PhoD signal sequence and others obtained from *B. subtilis* (e.g. Table I) by comparison to sequence databases (e.g. Table IV). The specification does not teach,

however, what alterations can be made to the *B. subtilis* PhoD signal sequence such that it will still function to direct translocation of a heterologous sequence across the cell membrane. Even for the known *B. subtilis* PhoD signal sequence, the specification describes experiments where it is shown that there is a unique requirement that the Tat proteins TatAd and TatCd be expressed in order for PhoD-dependent translocation across the cell membrane to occur in *B. subtilis* or *E. coli*. The amino acid residues of the *B. subtilis* PhoD signal sequence required for the TatAd/Cd interaction are not taught (e.g. page 33, first paragraph). The specification teaches that it is unclear why *B. subtilis* PhoD requires the presence of TatCd for efficient secretion, especially since the twin-arginine motif (R-R) for PhoD appears to be similar to other known R-R motifs (e.g. page 28).

State of the art/Predictability of the art: The prior art appears to be silent as to the use of a "PhoD" signal sequence to direct the secretion of a fusion protein across the cell membrane. Thus, the prior art does not offset the deficiencies of the instant specification with regard to providing a basis for one to envision other host cell/signal sequence combinations that will function to direct secretion of the desired heterologous sequence through the cell membrane barrier. Given that applicants' own data demonstrate that the *B. subtilis* PhoD signal sequence requires the expression of the *B. subtilis* TatAd and TatCd sequences, it would be unpredictable to attempt to practice the claimed invention in host cells that did not express the *B. subtilis* TatAd and TatCd proteins and did not use the *B. subtilis* PhoD signal sequence (i.e. the one shown in Table 1).

The amount of experimentation necessary: Given the combination of factors outlined above, including the complex nature of the invention and the great breadth of different host cell types and secretion signals encompassed by the rejected claims, and given the unpredictability with regard to practicing the claimed invention in the absence of the Tat Ad/Cd proteins that applicants' own data demonstrate are required for function, it would take undue, unpredictable experimentation to practice the claimed invention in the full, broadly claimed scope. Therefore, the instant specification is only found to be enabling for embodiments directed to bacterial cells wherein the *B. subtilis* PhoD sequence (i.e. that given in Table 1) and *B. subtilis* TatAd/Cd proteins are used to mediate secretion of the desired protein sequence.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gerald G Leffers Jr., PhD whose telephone number is (703) 308-6232. The examiner can normally be reached on 9:30am-6:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel can be reached on (703) 305-1998. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

GERRY LEFFERS
PRIMARY EXAMINER

Gerald G Leffers Jr., PhD
Primary Examiner
Art Unit 1636